

Changes in brain monoamines associated with agonistic interactions between males of *Eremobates marathoni* (Arachnida, Solifugae, Eremobatidae)

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Summary

Experiments were conducted to determine the effects of agonistic encounters between conspecific males on changes in monoamine concentrations in the brain of the solifuge *Eremobates marathoni* Muma & Brookhart. Serotonin (5-HT) levels were significantly reduced within 30 min after fighting in both dominant and subordinate animals as compared with isolated controls, and these differences persisted for up to 24 h. A similar decrease was found for octopamine (OA). In addition, 5-HT and OA were significantly lower in subordinate vs. dominant solifugids. Agonistic interactions had no effect on the concentrations of dopamine. This is the first demonstration of the possible role of monoamines in communicative displays and the establishment of social status in these arachnids.

Introduction

Over the past two decades there has been a concerted effort to analyse the role played by CNS neurotransmitters (NTs), comodulators, and neurohormones in the mediation of behaviour in vertebrates (Eichelman, 1987; Summers & Greenberg, 1995; Summers *et al.*, 1998; Punzo, 2001a). However, there have been significantly fewer studies on neurochemical parameters associated with behaviour in general, and agonistic interactions specifically, in arthropods and other invertebrates. For example, changes in levels of brain monoamines were shown to accompany a variety of ontogenetic shifts in behaviour in honeybee workers, including differential discrimination between various olfactory cues (Macmillan & Mercer, 1987) and the onset of nest-guarding behaviour (Moore *et al.*, 1987). Increases in the synthesis of protocerebral biogenic amines, RNA, protein, and acetylcholinesterase (AChE) have been shown to accompany learning in crustaceans (Punzo, 1985), insects (Lin & Roelofs, 1992; Punzo, 1996), and spiders (Punzo, 1988a).

With respect to the relationship between hormones, NTs, and the mediation of aggression and dominance status in arthropods, it was found that octopamine turnover rates increased significantly after fighting between conspecifics in the cricket *Gryllus bimaculatus* (Adamo *et al.*, 1995). Active defence of the nest against intruders was shown to be correlated with higher concentrations of octopamine (OA), dopamine (DA) and serotonin (5-HT) in the supraoesophageal ganglion (SEG) (brain) of worker honeybees (Harris & Woodring, 1992). It has been suggested that OA is part of a general arousal system which prepares insects and other arthropods for a variety of vigorous skeletomuscular activities, territorial defence, and helps the animal cope with stressful conditions (Corbet, 1991; Orchard

et al., 1993). An increase in 5-HT levels in the SEG has been implicated in the onset of flight behaviour in some beetles (Guerra *et al.*, 1991). Lobsters winning staged encounters (fighting bouts) with conspecifics exhibited a “dominant” posture that was accompanied by higher levels of 5-HT in various regions of the CNS when compared with subordinate animals (Kravitz, 1988).

The vast majority of studies concerning neurochemical correlates of behaviour in arthropods have been conducted on insects, while other taxa have received far less attention (Lin & Roelofs, 1992; Punzo, 1985, 1996). The solifugids (Arachnida, Solifugae), which have proved difficult to maintain in captivity (Punzo, 1998a), have been virtually ignored, especially with respect to their physiology and neurobiology.

Solifugids are well known for their pugnacious behaviour and voracious feeding habits (Muma, 1967; Punzo, 1998a, 2000). Both males and females exhibit a characteristic “threat display” when they are disturbed in any way (Cloudsley-Thompson, 1961; Muma, 1966; Punzo, 1998b, c). If one encounters another solifugid and/or a potential predator, the animal will typically stand its ground, open its massive chelicerae (jaws), lift itself via the three pairs of walking legs (legs II–IV) thereby elevating the body, and rock its body in an alternating backward and forward motion. After a variable period of time, the solifugid may either flee or strike at its antagonist. It is not unusual for the contest to escalate, resulting in the death of one or both combatants. Most male solifugids will exhibit this threat display even when confronted by a conspecific female before the onset of courtship.

The purpose of this study was to investigate neurochemical parameters associated with agonistic interactions between adult males of the solifuge *Eremobates marathoni*. Specifically, I was interested in whether or not there were any differences in the concentrations of monoamines (OA, DA, and 5-HT) in the SEG of dominant (winning) vs. subordinate (losing) males following agonistic encounters. To my knowledge, this is the first study to address neurochemical correlates of aggression in the Solifugae.

Methods

Animals and housing

Males were collected during June through August 1997 at the northern entrance to Big Bend National Park (28°37'N; 102°55'W; Brewster county, Texas; Chihuahuan Desert). A detailed description of the topography of this site as well as the dominant vegetation can be found elsewhere (Punzo, 2001b). Adult male solifugids (*Eremobates marathoni*) were abundant during this period and were easily found at night moving about on the surface between 2100–0200 h CST. Solifugids were collected manually and weighed to the nearest 0.1 g using a Ohaus Model 87 portable electronic balance (Central Scientific, Chicago, IL).

Solifugids were transported by van back to the laboratory within 72 h after collection, and housed

individually in holding cages as described elsewhere (Punzo, 1998b). The floor of each cage contained a layer of loose sand and an artificial hollowed "log" in which they could seek shelter. They were provided with water ad lib and fed three times per week on a mixed diet of crickets (*Gryllus* sp.), mealworm larvae (*Tenebrio molitor*) and cockroaches (*Periplaneta americana*). They were maintained at $22 \pm 1^\circ\text{C}$, 65% RH, and a 12:12 LD cycle, in a Percival Model 805 environmental chamber (Boone, IA). Animals were kept in these conditions for at least 2 weeks and then re-weighed on the day before the initiation of encounter trials. Since previous studies on arachnids have indicated that differences in body size may influence the outcome of agonistic encounters (Punzo, 2000), only males of approximately the same size (6.1–6.4 g) were used for encounter trials and subsequent neurochemical analyses. Voucher specimens have been deposited in the University of Tampa Invertebrate Collection.

Encounter trials

I used a rectilinear glass container ($26 \times 16 \times 12$ cm), divided in half by an opaque divider, as an encounter chamber to stage encounters between conspecific males as previously described (Punzo, 1998c). The floor of the chamber was provided with a layer of loose sand. All observations were conducted under Black lighting (Bio-Quip Inc., Model 2804, Gardena, California) behind a one-way mirror to minimise disturbance to the animals. I used a Sony 124L tape recorder to record verbal descriptions of each encounter. In addition, encounter trials were filmed with a Panasonic MVC-F81 video camera.

Before each encounter trial, one male solifuge (chosen at random) was placed at each end of the chamber, separated by the divider. A trial was initiated by removing the divider and allowing the animals to interact. Within a period of time ranging from 0.5–2.5 min over all trials, the combatants made contact with one of their first pair of walking legs. In solifugids, the first pair of walking legs (front legs) are elongated and used as sensory (tactile) organs and not for ambulatory movement (Punzo, 1998a). In a few cases ($n=7$), one or both of the solifugids would immediately attempt to flee after making initial contact with its opponent. These trials were not used in data analysis. In all other trials ($n=60$), following initial contact, both solifugids would typically face one another and move one or both of the front legs over the body of their opponent. During this sequence, each combatant exhibited the threat display described above (body elevated and moved in a backward–forward rocking motion, chelicerae opened). In all trials, this behaviour would continue until the fight escalated, with one or both solifugids attempting to bite the other before one of them backed away and tried to escape (usually by attempting to crawl up the sides of the chamber). A trial was considered terminated if at any time during the encounter one of the animals backed away and rapidly fled from the vicinity of its opponent (Punzo, 1998c). The solifugid that held its ground was

recorded as the "winner" (dominant animal), and the opponent that fled, the "loser" (subordinate). Each pair of combatants was subjected to only one encounter trial, following a protocol previously described for lizards (Summers & Greenberg, 1995). I conducted a total of 60 encounter trials, each consisting of 60 paired combatants.

Neurochemical analysis of brain tissue

Immediately following their designation as dominant or subordinate (based on the outcome of encounter trials), paired combatants were randomly assigned to one of three groups. Each group consisted of 40 solifugids (20 pairs). Solifugids in group 1 (G1) were anaesthetised with CO_2 thirty min after their encounter trials, and their brains (SEG) removed in a cold room as described previously (Punzo, 1993), weighed to the nearest 0.1 g, and frozen at -80°C for subsequent neurochemical analysis. Solifugids in G2 and G3 were anaesthetised and their brains removed and frozen at 24 h and 48 h, respectively, after encounter trials. This allowed me to determine not only what changes in monoamine concentrations, if any, might accompany male-male aggression, but also the rapidity of the response, and how long these changes might persist. The brains from another group of 20 solifugids (G4) maintained in isolation and not exposed to encounter trials were used as controls.

The SEGs from dominant, subordinate and control solifugids were analysed to determine the concentrations of those monoamines that have been identified as NTs, neuromodulators or neurohormones (5-HT, OA, DA) in arachnids (Meyer, 1991), insects (Orchard, 1982; Sombati & Hoyle, 1984; Downer & Hiripi, 1993), and crustaceans (Yeh *et al.*, 1996), using the protocol (high performance liquid chromatography with electrical detection; HPLC-ED, Beckman Model 47A) previously described in detail elsewhere (Punzo, 1994). To summarise, brains were removed from the head capsules as described by Punzo & Malatesta (1988). All glandular and fatty tissues were carefully removed from the surface of the brain samples. Individual brains were then weighed to the nearest 0.01 g in a Sartorius Model 54C electronic analytical balance. Each brain sample was placed in a 500 μl glass vial. Fifty μl of 200 mM perchloric acid were added and the sample homogenised. Following homogenisation, an additional 50 μl of perchloric acid were added to each vial. Samples were then centrifuged at 10,000 g for 3 min in a Sorvall Model 100A high speed centrifuge. Twenty μl of supernatant were injected directly into the HPLC column (40 cm in length, with a 0.2 μm pore diameter) packed with Hypersil and provided with a Hewlett-Packard 760E detector (0.40V). The mobile phase flow (flow rate 3000 psi) used to elute the monoamines consisted of 20 mM sodium acetate, 12% acetonitrile, 100 mM sodium dihydrogen orthophosphate, 0.3 mM EDTA disodium salt adjusted to pH 4.2, and 2.5 mM octane sulfonic acid. Each sample was compared with 5-HT, DA and OA

standards tested at the beginning of each assay run and retested at 30-min intervals. Monoamine concentrations (pmol/mg brain tissue) were expressed as means \pm SE.

Statistical analysis

All statistical procedures followed those described by Sokal & Rohlf (1995). Comparisons between mean concentrations of monoamines for the various groups were conducted using an analysis of variance (ANOVA), followed post-hoc by a Duncan's multiple range test. Significant differences between dominant and subordinate males following aggressive encounters were determined using an independent-samples *t*-test.

Results

Following an encounter, the dominant animals continued to exhibit the threat display while holding their ground for a period of time ranging from 0.5–1.5 min, before assuming a normal posture (with chelicerae closed and body lowered). The subordinate animals, which had been removed from the chamber immediately after attempting to flee (so that aggression could not escalate further), typically attempted to bite the plastic container used in their removal. However, when they were released onto the floor of their individual holding cages, they immediately sought refuge within the artificial log. In some cases, they did not leave their refuge for up to 24 h.

The data shown in Table 1 indicate that serotonin (5-HT) was the monoamine found in highest concentration in the brains (SEG) of isolated controls of *E. marathoni*, followed in decreasing order by OA and DA (Scheffe $F=3.14$, $p<0.01$).

The effects of agonistic interactions between conspecific males at various time intervals following encounter trials are also shown in Table 1. Levels of 5-HT after 30 min were significantly reduced in solifugids that "lost" encounters (subordinates) as compared with dominant animals ($t=3.91$, $p<0.05$) as well as with controls ($t=6.78$, $p<0.01$). This difference persisted for at least 24 h ($t=3.59$, $p<0.05$), with levels returning to normal after 48 h. Changes in 5-HT levels associated

with fighting occurred rapidly, since they were detected after only 30 min following an agonistic encounter.

A similar pattern was found for OA levels, which were also significantly lower in subordinate vs. dominant animals after 30 min ($t=3.67$, $p<0.05$) and 24 h ($t=3.03$, $p<0.05$). However, they returned to those levels associated with isolated controls within 48 h (Table 1). With respect to DA, no differences were found between solifugids exposed to agonistic encounters vs. controls at any time interval ($p>0.5$).

Discussion

Agonistic interactions between conspecifics are known to be associated with the synthesis and release of specific NTs, comodulators, and hormones associated with the stress response in numerous vertebrate taxa (Ansell & Bradley, 1973; Eichelman, 1987; Summers & Greenberg, 1995; Punzo, 2001a). Although much of the research initially focused on the role of hormones, such as testosterone and adrenocorticosteroids (Sapolsky, 1986; Greenberg & Crews, 1990), in the mediation of aggressive behaviour and social dominance, investigators have more recently turned their attention to the possible role of the CNS and various NTs (Summers *et al.*, 1998; Punzo, 2001a). For example, the social status of subordinate animals is associated with an increase in the utilisation of 5-HT in various brain regions from fish to mammals (Sapolsky, 1986; Haney *et al.*, 1990; Winberg *et al.*, 1997; Summers *et al.*, 1998). In addition, activation of brain catecholaminergic systems including DA, norepinephrine, and epinephrine is associated with increased levels of aggression and dominance in vertebrates (Mason, 1986; Haney *et al.*, 1990; Matter *et al.*, 1998). The results of the present study indicate that similar neurochemical parameters are associated with aggressive behaviour in arachnids.

Although the profile for monoamine concentrations in the SEG of *E. marathoni* (5-HT>OA>DA) is in general agreement with what little information is available on the neurochemistry of other arachnids (Meyer, 1991; Punzo, 1993, 1994), it should be pointed out that differences in monoamine profiles can vary between different arachnid orders and between different families within an order (Florey, 1967; Meyer, 1991; Punzo, 1988b, 1993).

The most pronounced changes in monoamine levels associated with reduced aggression involved 5-HT, occurring in subordinate males within 30 min of an agonistic encounter. Levels of 5-HT were also reduced in dominant males as compared with isolated controls. A similar reduction in OA levels was also observed in dominant males. This is interesting in view of earlier reports that OA and 5-HT often work antagonistically (Bicker & Menzel, 1989). Thus, the act of fighting (and the stress associated with it) in male solifugids resulted in a decrease in SEG serotonin levels. This is the first demonstration of an association between monoaminergic activity and aggression in solifugids. Changes in serotonin have been previously associated with aggressive behaviour in crustaceans (Kravitz, 1988; Bicker &

Monoamine	Controls	Time after encounter		
		30 min	24 h	48 h
Serotonin (5-HT)	54.8 (6.5)			
Subordinate		36.1 (2.1)	36.9 (0.9)	56.2 (2.3)
Dominant		46.1 (1.2)	45.4 (1.8)	52.5 (2.6)
Octopamine	23.2 (5.4)			
Subordinate		10.7 (0.5)	16.9 (0.4)	21.7 (1.4)
Dominant		17.4 (0.7)	21.9 (0.8)	23.8 (1.8)
Dopamine	13.3 (3.5)			
Subordinate		13.9 (1.1)	15.2 (0.7)	13.9 (0.6)
Dominant		14.2 (0.5)	13.6 (1.1)	12.7 (0.7)

Table 1: Concentrations of various monoamines (pmol/mg) in the supraoesophageal ganglion (brain) of isolated controls and subordinate and dominant males of *Eremobates marathoni* following agonistic encounters. Data expressed as means (\pm SE). See text for details.

Menzel, 1989; Yeh *et al.*, 1996). The injection of 5-HT into lobsters and crayfish caused them to flex their tails, a response associated with a display of dominance in this group (Yeh *et al.*, 1996).

The present study demonstrates that lower levels of aggression and dominance in solifugids are associated with changes in monoamines in the brain that are similar to those that have been well established for vertebrates. A variety of stimuli, including social interactions, activate neuroendocrine stress mechanisms in vertebrates, which are thought to be mediated by changes in CNS neurotransmitters brought about primarily by the activation of monoaminergic systems (Ansell & Bradley, 1973; Eichelman, 1987; Summers *et al.*, 1998). For example, 5-HT levels decreased significantly after one h and one day in the brain (diencephalon and non-optic lobe midbrain) of the lizard *Anolis carolinensis* after losing aggressive encounters (Summers & Greenberg, 1995). Similarly, no changes were detected for DA levels over these time intervals. However, subordinate males had significantly lower DA levels after one week as compared with subordinates after one h. In another study, changes in serotonergic content and turnover between individuals of different social status were found in the telencephalon and diencephalon of territorial vs. satellite males in the lizard *Sceloporus jarrovi* (Matter *et al.*, 1998). In addition, levels of 5-HT in the telencephalon and diencephalon were found to decrease significantly following male-male aggression in rodents (Haney *et al.*, 1990) and fish (Winberg *et al.*, 1992, 1997).

Octopamine levels also decreased significantly in males of *E. marathoni* following agonistic encounters. This is in agreement with previous work indicating that OA appears to be central to the elicitation of the overall arousal response of arthropods (Kravitz, 1988; Corbet, 1991), and elevated OA activity has been shown to accompany stress (Harris & Woodring, 1992; Downer & Hiripi, 1993), increased locomotor activity (Orchard *et al.*, 1993; Adamo *et al.*, 1995), and a wide range of systemic physiological responses including respiration, gastrointestinal peristalsis, glycogenolysis, cardioacceleration, osmoregulation, and pheromone production in insects (Corbet, 1991). It has been further suggested that certain behaviour patterns can be elicited by the activation of specific octopaminergic pathways in arthropods, an idea known as the "orchestration hypothesis" (Sombati & Hoyle, 1984). For example, administration of exogenous OA triggered diurnal hyperactivity in nocturnal moths (Shimizu & Fukamii, 1981). Changes in OA levels in the CNS have been associated with ontogenetic shifts in specific behavioural acts in solifugids (Punzo, 1994) and social insects (Bicker & Menzel, 1989; Brandes *et al.*, 1990), and an increase in OA activity was found in the brain of the cricket following aggressive interactions between conspecifics (Adamo *et al.*, 1995).

Although most of the research on OA has focused on insects, some previous studies, including the present one, suggest that this monoamine plays an important role in regulating the behaviour of other arthropods as well. For example, the escape behaviour of crayfish, which requires a tail flip response, is enhanced by OA (Bicker

& Menzel, 1989). The injection of OA into freely moving lobsters elicited submissive postural responses toward conspecifics (Kravitz, 1988). The application of OA caused engorged ticks to detach from their hosts (Mason, 1986). For OA, direct comparison with vertebrates is not possible since OA has not been identified as an NT in this group.

In summary, significant changes in concentrations of 5-HT and OA in the SEG were found to be associated with agonistic behaviour in male solifugids. It has been well established that 5-HT is an NT involved in the mediation of dominance and aggression in vertebrates, and the results of this study suggest that 5-HT and OA concentrations in the SEG are important for the establishment of social status and the elicitation of communicative displays in arthropods as well. Future endeavours should assess the role of monoaminergic systems in other species of solifugids as well as other arachnid and arthropod taxa in order to determine if similar changes in monoamine profiles accompany aggressive behaviour in these groups.

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